Dual-Electrode Electrochemical Detection for Poly(dimethylsiloxane)-Fabricated Capillary Electrophoresis Microchips

R. Scott Martin, Andrew J. Gawron, and Susan M. Lunte*

Department of Pharmaceutical Chemistry, and Center for Bioanalytical Research, The University of Kansas, 2095 Constant Avenue, Lawrence, Kansas 66047

Charles S. Henry

Department of Chemistry, Mississippi State University, 118 Hand Laboratory, Mississippi State, Mississippi 39762

The development of a poly(dimethylsiloxane)-based (PDMS-based) microchip electrophoresis system employing dual-electrode electrochemical detection is described. This is the first report of dual-electrode electrochemical detection in a microchip format and of electrochemical detection on chips fabricated from PDMS. The device described in this paper consists of a top layer of PDMS containing the separation and injection channels and a bottom glass layer onto which gold detection electrodes have been deposited. The two layers form a tight reversible seal, eliminating the need for high-temperature bonding, which can be detrimental to electrode stability. The channels can also be temporarily removed for cleaning, significantly extending the lifetime of the chip. The performance of the chip was evaluated using catechol as a test compound. The response was linear from 10 to 500 μM with an LOD (S/N = 3) of 4 μM and a sensitivity of 45.9 pA/μM. Collection efficiencies for catechol ranged from 28.7 to 25.9% at field strengths between 200 and 400 V/cm. Dual-electrode detection in the series configuration was shown to be useful for the selective monitoring of species undergoing chemically reversible redox reactions and for peak identification in the electropherogram of an unresolved mixture.

Capillary electrophoresis (CE) in the microchip format is an emerging technology that has generated a great deal of interest. The advantages of microchip CE include fast analysis times, portability, disposability, consumption of minute amounts of solvent, the possibility of electrokinetic control of fluids, and the use of high separation field strengths.[1,2] Many different procedures and substrates have been investigated for the fabrication of microchip CE devices. In the initial studies, chips were constructed using glass or quartz, since these substrates are optically transparent and exhibit electroosmotic flow properties similar to those of

fused silica.[3−6] Glass chips were constructed using modifications of procedures used to manufacture silicon chips. Disadvantages of glass devices include the use of hydrofluoric acid for etching, difficulties in bonding a cover plate to the device, and the fragile nature of the final device.

More recently, alternative materials including plastics[7−9] and low-temperature cofired ceramics (LTCC) were investigated for microchip CE systems.[10] The ease of fabrication and relatively low cost of devices manufactured from these materials have made them an attractive alternative to optical-quality glass. In particular, there has been a growing interest in the application of poly(dimethylsiloxane)-based (PDMS-based) microchip CE devices.[11,12] PDMS is a hydrophobic elastomer in which channels can be formed by curing the polymer over a master that contains the desired microchip features. Several groups have demonstrated electroosmotic flow (EOF)- and CE-based separations in PDMS microchip devices.[11−15] A major advantage of PDMS is that multiple (30 or more) devices can be produced rapidly from a single master with only minimal use of clean room facilities.[14]

---

* Corresponding author. Phone: (785) 864-3811. Fax: (785) 864-5736. E-mail: slunte@ukans.edu.

(1) Dolnik, V.; Liu, S.; Jovanovich, S. *Electrophoresis* **2000**, *21*, 41−54.

(2) *Micro Total Analysis Systems '98*; Harrison, D. J., van den Berg, A., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 1998.

(3) Harrison, D. J.; Manz, A.; Fan, Z.; Lüdi, H.; Widmer, H. M. *Anal. Chem.* **1992**, *64*, 1926−1932.

(4) Effenhauser, C. S.; Manz, A.; Widmer, H. M. *Anal. Chem.* **1993**, *65*, 2637−2642.

(5) Jacobson, S. C.; Hergenroder, R.; Koutny, L. B.; Ramsey, J. M. *Anal. Chem.* **1994**, *66*, 1114−1118.

(6) Effenhauser, C. S.; Paulus, A.; Manz, A.; Widmer, H. M. *Anal. Chem.* **1994**, *66*, 2949−2953.

(7) Becker, H.; Gartner, C. *Electrophoresis* **2000**, *21*, 12−26.

(8) Martynova, L.; Locascio, L. E.; Gaitan, M.; Kramer, G. W.; Christensen, R. G.; MacCrehan, W. A. *Anal. Chem.* **1997**, *69*, 4783−4789.

(9) Rossier, J. S.; Schwarz, A.; Reymond, F.; Ferrigno, R.; Bianchi, F.; Girault, H. H. *Electrophoresis* **1999**, *20*, 727−731.

(10) Henry, C. S.; Zhong, M.; Lunte, S. M.; Kim, M.; Bau, H.; Santiago, J. *Anal. Commun.* **1999**, *36*, 305−308.

(11) McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H.; Schueller, O. J. A.; Whitesides, G. M. *Electrophoresis* **2000**, *21*, 27−40.

(12) Ocvirk, G.; Munroe, M.; Tang, T.; Oleschuk, R.; Westra, K.; Harrison, D. J. *Electrophoresis* **2000**, *21*, 107−115.

(13) Chan, J. H.; Timperman, A. T.; Qin, D.; Aebersold, R. *Anal. Chem.* **1999**, *71*, 4437−4444.

(14) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974−4984.

(15) Effenhauser, C. S.; Bruin, G. J. M.; Paulus, A.; Ehrat, M. *Anal. Chem.* **1997**, *69*, 3451−3457.

While there have been a large number of papers regarding the construction and evaluation of microchip CE devices, very few investigations of alternate detection modes have been reported. The most popular mode of detection for microchip CE as well as the primary detection mode used thus far with PDMS microchip devices is laser-induced fluorescence (LIF).[1,11] LIF provides the necessary sensitivity and small sampling area needed to detect analytes in these miniaturized systems. However, LIF detection typically requires derivatization of the sample with a fluorophore either before or after the separation has taken place. In addition, only a select number of wavelengths can be used for excitation. Mass spectrometry (MS) has also been used as a detection mode for both glass[1,16,17] and PDMS devices.[13] While MS provides more chemical information than any other detection method, commercially available systems are not inherently portable, as they are much larger than the chips, and MS is more costly and less sensitive than LIF.

Electrochemical (EC) detection has been demonstrated as an attractive alternative with both conventional[18–22] and microchip CE.[9,10,23–27] Many compounds can be detected without derivatization, and the sensitivity of EC detection is comparable to that of fluorescence detection. EC detection is ideally suited for the microchip format because the microelectrodes can be fabricated using many of the same procedures that are used to construct microchips. Since electrochemical detection is based on the redox reaction occurring at the electrode surface and not on an optical path length, it can be miniaturized without loss in sensitivity. With concomitant miniaturization and integration of the power supplies and potentiostats, a truly self-contained micro total analysis system (μ-TAS) based on CE-EC can be envisioned.

Several groups have reported EC detection for microchip CE. Gavin and Ewing originally demonstrated EC detection for separations in open-channel electrophoresis chips by using Pt microelectrode arrays for continuous monitoring of analytes that had been sampled by a capillary.[23,24] In 1998, Woolley and co-workers reported the development of a microchip CE-EC system that employed etched channels and Pt electrodes on a glass substrate. This device was used to separate and detect neurotransmitters and DNA.[25] Subsequently, our group reported a microchip CE-EC system composed of LTCC using a 25 μm wire as the working electrode.[10] The LTCC chips were relatively inexpensive to fabricate and exhibited electroosmotic properties similar to those of glass. Recently, Wang and co-workers reported two different microchip CE-EC systems—one using a sputtered Au electrode[26] and a second using an externally mounted screen-printed electrode.[27] The use of the externally mounted screen-printed electrode resulted in detection limits substantially lower than those previously reported with microchip CE-EC systems. Rossier and co-workers reported the initial use of EC detection in plastic microchip CE devices using electrodes made from carbon ink.[9]

In most cases, selectivity in electrochemical detection is achieved by a judicious choice of the potential of the working electrode. However, there are often situations where greater selectivity is desired, for example, in the analysis of complex biological samples. This can be achieved by using chemically modified electrodes and multiple-electrode systems. The use of two electrodes in series for the selective detection of compounds exhibiting chemically reversible redox reactions has been demonstrated previously for conventional CE.[28–33] This methodology has been employed for the determination of many compounds including catecholamines, phenolic acids, thiols, and disulfides.

In this paper, we describe electrochemical detection in a PDMS microchip CE device. The microchip CE-EC system utilizes gold band electrodes that are deposited on a glass substrate. To the best of our knowledge, this is the only documented account of CE separations using mixed PDMS/glass devices. In addition, we report the first example of a dual-electrode detector for microchip CE utilizing two gold working electrodes configured in series. An example of the selectivity that can be gained by this electrochemical detection mode is demonstrated using catechol and ascorbic acid as model compounds.

EXPERIMENTAL SECTION

Chemicals. The following chemicals and materials were used as received: SU-8 50 photoresist (MicroChem Corp., Newton, MA), propylene glycol methyl ether acetate (Aldrich), 3-in. silicon wafers (test grade, Silicon Inc., Boise, ID), tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (United Chemical Technologies, Bristol, PA), H_2O_2 (30%) and Sylgard 184 (Fisher Scientific), sodium-lime glass (Valley Design Corp., Westford, MA), float-glass (Kennedy Glass, Lawrence, KS), chromium-plated tungsten rods and tungsten boats (Kurt J. Lesker Co., Clairton, PA), American gold eagle coin (U.S. Mint), AZ P4330-RS photoresist, AZ 400K developer, and AZ 300T photoresist stripper (Clariant Corp., Somerville, NJ), CR-7S chromium etchant (Cyantek Corp., Fremont, CA), and catechol, 5-hydroxyindole-3-acetic acid (HIAA), hydroquinone, ascorbic acid, and tyrosine (Sigma).

Fabrication. *PDMS Structures.* The method used to create channels in PDMS is based on previously published procedures.[14,34] Briefly, to create masters for PDMS device construction, a 3-in. silicon wafer was coated with SU-8 50 negative photoresist using a spin coater (Brewer Science, Rolla, MO) operating at 2500 rpm for 20 s. After a preexposure bake, the coated wafer was exposed to light via a near-UV flood source (Autoflood 1000, Optical Associates, Milpitas, CA) through a negative chrome mask (LSI Photomask, Chandler, AZ) that contained the desired channel

(16) Figeys, D.; Gygi, S. P.; McKinnon, G.; Aebersold, R. *Anal. Chem.* **1998**, *70*, 3728–3734.
(17) Figeys, D.; Aebersold, R. *Anal. Chem.* **1998**, *70*, 3721–3727.
(18) Wallingford, R. A.; Ewing, A. G. *Anal. Chem.* **1987**, *59*, 1762–1766.
(19) Holland, L. A.; Lunte, S. M. *Anal. Commun.* **1998**, *35*, 1H–4H.
(20) O'Shea, T. J.; Weber, P. L.; Bammel, B. P.; Lunte, C. E.; Lunte, S. M.; Smyth, M. R. *J. Chromatogr.* **1992**, *608*, 189–195.
(21) Voegel, P. D.; Zhou, W.; Baldwin, R. P. *Anal. Chem.* **1997**, *69*, 951–957.
(22) Zhong, M.; Lunte, S. M. *Anal. Chem.* **1996**, *68*, 2488–2493.
(23) Gavin, P. F.; Ewing, A. G. *J. Am. Chem. Soc.* **1996**, *118*, 8932–8936.
(24) Gavin, P. F.; Ewing, A. G. *Anal. Chem.* **1997**, *69*, 3838–3845.
(25) Woolley, A. T.; Lao, K.; Glazer, A. N.; Mathies, R. A. *Anal. Chem.* **1998**, *70*, 684–688.
(26) Wang, J.; Tian, B.; Sahlin, E. *Anal. Chem.* **1999**, *71*, 3901–3904.
(27) Wang, J.; Tian, B.; Sahlin, E. *Anal. Chem.* **1999**, *71*, 5436–5440.

(28) Zhong, M.; Lunte, S. M. *Anal. Chem.* **1999**, *71*, 251–255.
(29) Holland, L. A.; Lunte, S. M. *Anal. Chem.* **1999**, *71*, 407–412.
(30) Holland, L. A.; Harmony, N. M.; Lunte, S. M. *Electroanalysis* **1999**, *11*, 1–4.
(31) Zhong, M.; Lunte, S. M. *Anal. Commun.* **1998**, *35*, 209–212.
(32) Zhong, M.; Zhou, J.; Lunte, S. M.; Zhao, G.; Giolando, D. M.; Kirchhoff, J. R. *Anal. Chem.* **1996**, *68*, 203–207.
(33) Lin, B. L.; Colon, L. A.; Zare, R. N. *J. Chromatogr., A* **1994**, *680*, 263–270.
(34) Duffy, D. C.; Gillis, H. L.; Lin, J.; Sheppard, N. F.; Kellogg, G. J. *Anal. Chem.* **1999**, *71*, 4669–4678.

features. Following a postexposure bake, the wafer was developed in propylene glycol methyl ether acetate. The thickness of the positively raised structures, which is equal to the subsequent depth of the channels in the PDMS structures, was measured with a profilometer (Alpha Step-100, Tencor Instruments, Mountain View, CA). The master was silanized by placing it in a desiccator under vacuum for 1 h along with a vial containing a few drops of silanizing agent (tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane). A 10:1 mixture of PDMS oligomer and cross-linking agent (Sylgard 184), which had been degassed under vacuum, was poured onto the master. After at least 1 h of curing at 65 °C, the PDMS was removed from the mold to yield a pattern of negative relief channels in the PDMS. Channel reservoirs were cut out with a hole punch, and the rest of the PDMS was trimmed to size with a razor blade.

Electrode Plates. Soda-lime glass squares (2.5 in.^{2} × $^1/_4$ in.) and float-glass squares (2.5 in.^{2} × $^3/_{32}$ in.) were used as electrode substrates. The squares were first chemically cleaned by immersion in piranha solution (7:3 H_2SO_4:H_2O_2) for at least 15 min to remove organic impurities. (***Caution!*** *Piranha solution is a powerful oxidizing agent that reacts violently with organic compounds; it should be handled with extreme care.*) The squares were rinsed with copious amounts of deionized water and then with IPA, dried in a stream of Ar, and placed in an oven at 105 °C for 5 min. At this point, the squares were ready for the Cr/Au deposition and were transferred directly into a thermal evaporator (Consolidated Vacuum Corp., Rochester, NY) equipped with two electrode blocks and a cryogenic vacuum pump (CTI-Cyrogenics, Mansfield, MA). After pump-down, the thermal evaporator was operated at a base pressure of 2×10^{-9} Torr. All metal thicknesses and deposition rates were measured with a quartz crystal deposition monitor (Inficon XTM/2, Leybold Inficon, Syracuse, NY). Chromium was evaporated by using chromium-coated tungsten rods; approximately 50 Å of Cr was deposited at 1 Å/s. Gold was deposited by using gold pieces (~0.5 cm) cut from a gold coin source that were placed in a tungsten boat; approximately 2000 Å of Au was deposited at 5 Å/s. Positive photoresist (AZ P4330-RS) was applied to the Cr/Au-coated plate that was spinning at 200 rpm; after application was complete, the spin coater was ramped to 3000 rpm for 30 s. After a preexposure bake, the coated Cr/Au plate was exposed to near-UV light via the UV flood source through a positive mask that contained the desired electrode features. The positive mask was made from a computer design drawn in Freehand (PC version 8.0, Macromedia, Inc., San Francisco, CA); it was transferred onto a transparency using an image setter with a resolution of 2400 dpi by a commercial printing service (Lasergraphics, Lawrence, KS). The plates were developed and the photoresist patterns cured by postexposure baking. The photoresist that remains after this procedure protects the underlying Cr/Au layers from the subsequent etching steps and defines the eventual electrode features. The plates were placed in a 1:1 aqua regia:water solution to etch the gold layer. Following a water rinse, the Cr layer was removed in chromium etchant solution and the photoresist was removed in photoresist stripper, leaving behind the intact Cr/Au electrodes. The electrode plates were then rinsed consecutively with water and IPA, dried under a stream of Ar, and stored in a desiccator until used.

Construction of PDMS/Electrode Plate Devices. The PDMS layer, which contains channels and reservoirs, and the electrode
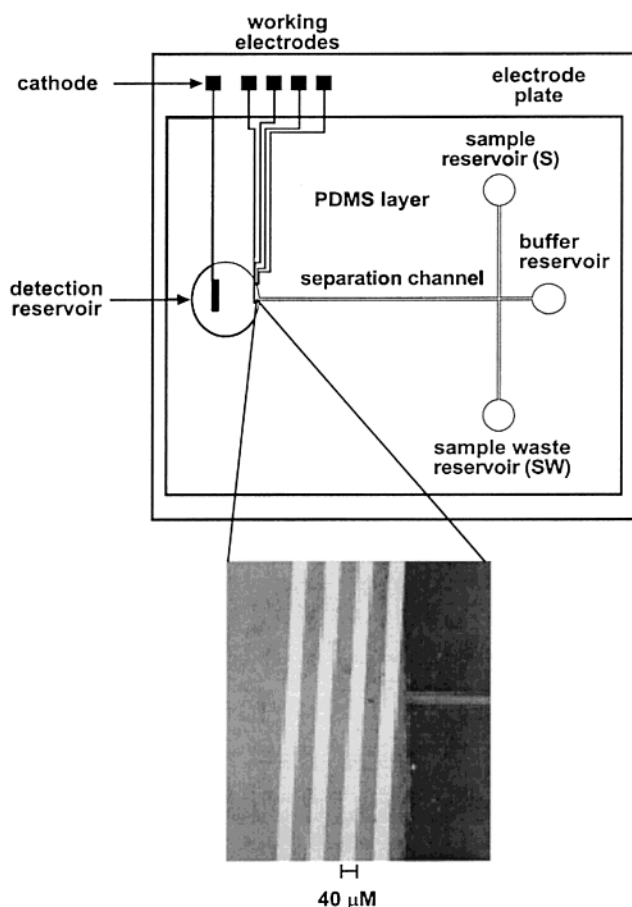

Figure 1. Microchip CE-EC schematic. Separation channel dimensions: 25 μm wide, 50 μm deep, 3.3 cm from injection T to detection reservoir, 0.1 cm from injection T to buffer reservoir. Sample channel dimensions: 25 μm wide, 50 μm deep, 1 cm from S to injection T, 1 cm from SW to injection T. Electrode dimensions: 40 μm wide, 40 μm apart. The first electrode is 15 μm from the channel exit. The reference and auxiliary electrodes (not shown) are located in the detection reservoir.

plate were reversibly bonded together by cleaning both plates with IPA, drying under a stream of N_2, and aligning the first working electrode on the electrode plate close to the end of the separation channel (10–20 μm; see Figure 1) with the aid of a light microscope. Once the alignment was performed, air bubbles between the two layers were removed using pressure until the device was sealed.

Electrophoresis Procedures. Capillary zone electrophoresis separations were carried out in uncoated channels using a Spellman CZE 1000R high-voltage power supply (Spellman High Voltage Electronics, Hauppauge, NY). (***Caution!*** *To avoid electrical shock, the high-voltage power supply should be used with extreme care.*) The electrophoresis buffer consisted of either 15 or 25 mM boric acid, pH 9.2. Degassed and filtered buffer was introduced into the reservoirs and flushed through the channels under vacuum until air bubbles were no longer observed under a microscope. The device was conditioned with run buffer for 15 min at field strengths between 150 and 300 V/cm. Stock solutions of hydroquinone (10 mM), catechol, and HIAA (1 mM) were prepared daily in deionized water; stock solutions of ascorbic acid and tyrosine (1 mM) were made weekly in deionized water. Appropriate dilutions were made prior to use; all solutions introduced into reservoirs had been degassed and filtered.

Injection was carried out by applying a high voltage to the sample reservoir for 1 s with both the sample waste and detection reservoirs grounded and the buffer reservoir left floating (Figure 1). In most experiments, the injection voltage was equal to the separation voltage (V_s), except in experiments in which collection efficiencies were determined. In this case, a constant injection voltage of 1050 V was used with various V_s's. Injection volumes were not estimated because of previously reported problems with sample dispersion into the separation channel when this type of injection scheme was used.[26] Once injection was complete, the V_s was applied to the buffer reservoir, and the separation was carried out with the detection reservoir grounded.

Electrochemical Detection. Amperometric detection was performed in either a three-electrode (for single-electrode detection) or a four-electrode (for dual-electrode detection) format, with each working electrode being controlled by a separate potentiostat (LC-4CE, Bioanalytical Systems, West Lafayette, IN). A small square of perforated PDMS was reversibly sealed around the detection reservoir to increase its capacity. To this reservoir were added a Pt wire auxiliary electrode and an Ag/AgCl reference electrode (RE-6, Bioanalytical Systems), with the auxiliary electrode being placed as close to the channel exit as possible. The first working electrode (E_1) was maintained at a potential of either +750 or +780 mV (vs Ag/AgCl). In experiments in which dual-electrode detection was employed, the second working electrode (E_2) was held at a potential of −100 mV (vs Ag/AgCl). The analogue signal from the amperometric detector was converted to a digital signal with an analog−digital converter (DA-5, Bioanalytical Systems) and collected with the aid of the accompanying software. Cleaning of the working electrodes was accomplished by applying a bipolar square wave of ±1.8 V at 30 Hz for 30 s to E_1 while buffer was electrokinetically pumped through the separation channel.

RESULTS AND DISCUSSION

Physical Characteristics and Operating Parameters. Previous reports concerning microchip CE-EC devices have involved the use of a single substrate—glass,[23−27] ceramic,[10] or plastic.[9] It is not possible to fabricate rugged, stable metal electrodes on PDMS because of its pliable nature. Therefore, in the devices reported here, the separation and injection channels were imprinted into the PDMS, while the gold detection electrodes were fabricated on a glass plate. As can be seen in Figure 1, the electrode plates were fabricated with four working electrodes; this permits the continued use of a plate if one or more of the electrodes fail.

As with conventional CE-EC, one of the critical issues with microchip CE-EC is decoupling the separation voltage from the electrochemical detector. In this case, end-column detection was employed.[35] Alignment of the electrodes was accomplished by carefully placing the PDMS layer over the electrode plate so that the electrodes were just outside, yet as close as possible to, the exit of the separation channel. Using this approach, the first electrode was approximately 15 (±5) μm from the end of the separation channel. While this distance was sufficient to dissipate the separation voltage, it did cause some band broadening. However, if the electrodes were placed too close to the channel

exit (5 μm or closer), the electric field caused a large increase in noise, making sensitive detection of analytes impossible.

The binding of PDMS to glass can be accomplished in either a reversible or an irreversible fashion. EOF- and CE-based separations have been shown in both reversibly[12] and irreversibly[11,14] bonded PDMS microfabricated devices. In the reversibly bonded devices, a PDMS layer that contains separation and injection channels is simply pressed onto and subsequently sealed against another slab of PDMS or glass. If both surfaces are oxidized in a plasma cleaner prior to bonding, the seal is irreversible and the two layers cannot be separated. Irreversibly bonded devices are leak-free and can withstand pressures up to 50 psi. However, the EOF steadily increases after 3 days of use and the channels cannot be regenerated if they become clogged.[11] Reversibly bonded devices are stable for long periods of time (4 months) and are easy to clean if the channel becomes blocked; however, these devices cannot withstand pressures greater than 5 psi and are more prone to leakage.[12]

All of the devices used in this study were constructed using the reversible bonding procedure. Once the PDMS layer was sealed onto the glass electrode plate, the chip was extremely sturdy due to the added rigidity of the glass plate. If one of the working electrodes became passivated or if blockage and/or air bubbles occurred in the channels, the PDMS could simply be removed, cleaned, and resealed 15 μm from a functioning electrode on the same electrode plate. Hence, the lifetime of a chip was dependent solely upon the longevity of the electrode plate because the same PDMS layer could be used many times.

Hydroquinone was used as a neutral marker to evaluate EOF in the microchip device. Using 15 and 25 mM boric acid buffer at pH 9.2 led to calculated EOF's of $(3.60 \pm 0.03) \times 10^{-4}$ cm²/(V s) ($n = 5$) and $(2.94 \pm 0.03) \times 10^{-4}$ cm²/(V s) ($n = 5$), respectively. These values compare well to the previous published values for reversibly sealed PDMS devices,[12] indicating that the microchip supports cathodic EOF.

Electrode stability can be a concern in microchip devices that will be used for multiple runs or long periods of time. The lifetime of bilayer metal electrodes is affected by grain boundary diffusion of the metal adhesion layer through the overlying metal layer. This diffusion leads to the formation of an insulating oxide layer on the surface of the electrode and subsequent electrode failure.[36] The extreme potentials and/or high temperatures required for the bonding of glass chips can increase the rate of grain boundary diffusion[36−38] and limit electrode life. In the procedure described here, bonding occurs at room temperature and the gold working electrodes were found to be quite rugged. The typical lifetime of one continuously operated working electrode was found to be approximately 150 separations over an 8-h period, although one electrode was used for greater than 300 separations over a 12-h period before failure was finally observed. Again, if one electrode failed, the PDMS could be resealed next to a functioning electrode on the same electrode plate.

(35) Sloss, S.; Ewing, A. G. *Anal. Chem.* **1993**, *65*, 577−581.

(36) Anderson, J. L.; Winograd, N. In *Laboratory Techniques in Electroanalytical Chemistry*; Kissinger, P. T., Heineman, W. R., Eds.; Marcel Dekker: New York, 1996; pp 333−366.

(37) Goss, C. A.; Charych, D. H.; Majda, M. *Anal. Chem.* **1991**, *63*, 85−88.

(38) Sreenivas, K.; Reaney, I.; Maeder, T.; Setter, N.; Jagadish, C.; Elliman, R. G. *J. Appl. Phys.* **1994**, *75*, 232−239.
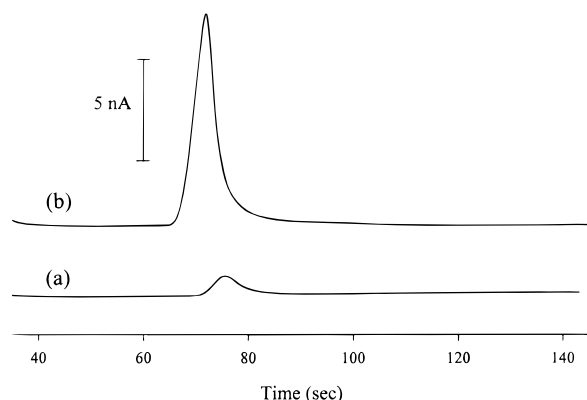
Figure 2. Effect of a bipolar square waveform on the response for single-electrode detection of 500 μM catechol (1 mM stock solution diluted 1:1 with run buffer): (a) response after 25 consecutive runs; (b) response after application of the bipolar square waveform to the detection electrode. Separation conditions: 15 mM boric acid, pH 9.2; applied potential 870 V (250 V/cm). Injection: 1 s (S to SW) at 870 V. $E(E_1) = +780$ mV vs Ag/AgCl.

Electrode fouling is also a potential concern in microchip systems where multiple samples will be analyzed. Analysis of 25 consecutive samples of varying concentrations of catechol resulted in a substantial reduction in electrode response (Figure 2a). This fouling was reversed by the application of a bipolar square wave to the working electrode (Figure 2b). As can be seen by this figure, pretreatment of the electrode surface restored the electrode response. In a separate experiment utilizing a 200 μM catechol solution (data not shown), it was determined that application of the bipolar square wave to a fouled electrode restored 98% of the original electrode response. However, although the cleaning procedure improved the overall performance of the electrodes, it decreased their overall life span. Repeated applications (>10) of the bipolar waveform eventually destroyed the electrodes, although optimization of the applied square-wave potential was not attempted in this study.

Sample Injection and Peak Stacking. In these studies, samples were injected using a basic T injector design (Figure 1). Previous reports concerning injection for microchip CE have emphasized that strict voltage control of the injection volume is necessary to prevent leakage into the separation channel.[39] The most reproducible injections are accomplished by shaping the injection plugs with voltage control from four directions and applying "pushback voltages" to prevent leakage from side channels. With EC detection, it is impossible to apply a voltage to the detection reservoir because the detector must be at ground. Woolley and co-workers were able to circumvent this problem by continuously applying separate voltages to the buffer, sample, and sample waste reservoirs.[25]

In this work, injections were made directly into the separation channel using a modified version of the procedure that was previously reported by Wang and co-workers.[26,27] Although this injection methodology does not involve the use of strict voltage control over each reservoir, reproducible injections were obtained by use of field-amplified stacking. The injection of a solution of 200 μM catechol diluted in water resulted in a response and an
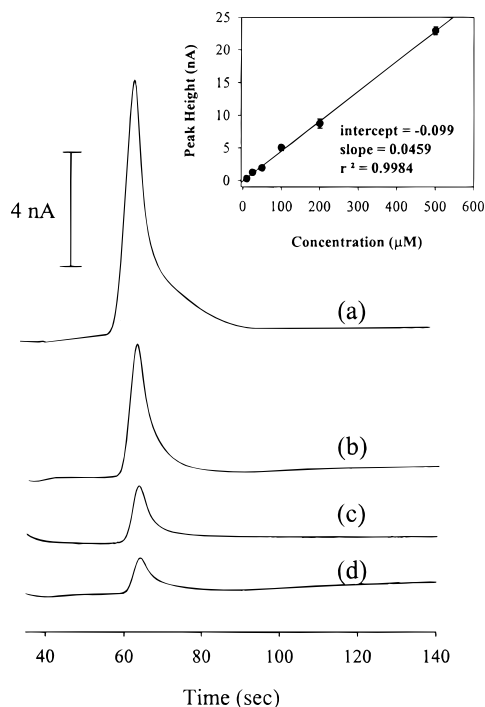
(39) Shultz-Lockyear, L. L.; Colyer, C. L.; Fan, Z. H.; Roy, K. I.; Harrison, D. J. *Electrophoresis* **1999**, *20*, 529−538.

Figure 3. Electropherograms for (a) 200, (b) 100, (c) 50, and (d) 25 μM catechol. The inset graph shows a linear range of 10−500 μM and a sensitivity of 45.9 pA/μM. Separation conditions: 15 mM boric acid, pH 9.2; applied potential 870 V (250 V/cm). Injection: 1 s (S to SW) at 870 V. $E(E_1) = +780$ mV.

efficiency of 8.73 ± 0.71 nA ($n = 3$) and 1037 ± 104 plates (31 433 $\pm$ 3166 plates/m; $n = 3$), respectively. This stacking protocol was used in all subsequent studies. These efficiencies are far from optimal, and investigation into band-broadening contributions from sources other than our injection protocol, such as PDMS adsorption and/or the use of end-column detection, is currently under investigation. Ocvirk et al.[12] and Woolley et al.[25] have described band-broadening contributions from PDMS adsorption and end-column detection in microchip devices, respectively.

The response for catechol was found to be linear between 10 and 500 μM with a correlation coefficient of 0.9984, a sensitivity of 45.9 pA/μM, and an average migration time of 63 ± 2 s ($n = 18$). Figure 3 shows four electropherograms for varying concentrations of catechol diluted with water. The electrode surface was pretreated by application of the bipolar square wave between each injection of a different catechol concentration. On the basis of the average response generated from 10 μM catechol (S/N = 8), the limit of detection (LOD) for catechol diluted in water was estimated to be 4 μM at a S/N = 3. This LOD is approximately 1 order of magnitude higher than that in previous work utilizing a similarly configured conventional CE-EC system for the detection of catecholamines[22] but is 3 times lower than the LOD obtained for catechol using glass microchip CE-EC devices that employ microfabricated metal working electrodes.[25]

Dual-Electrode Detection. All previously reported closed-channel microchip devices with EC detection have employed single-electrode detection.[9,10,25−27] Dual-electrode detection has several advantages, including increases in selectivity and sensitivity for compounds undergoing chemically reversible redox reactions. This detection mode is especially useful for the analysis of complex samples, where a high detection potential can lead to a
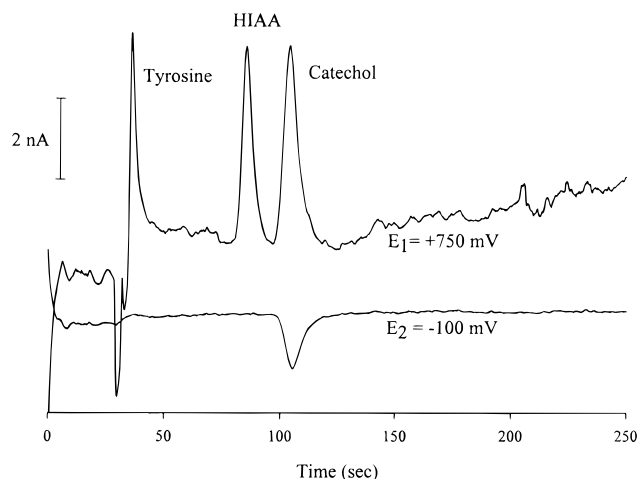
Figure 4. Dual-electrode detection in a series configuration using Au/Cr microelectrodes for 100 μM tyrosine, HIAA, and catechol. Separation conditions: 25 mM boric acid, pH 9.2; applied potential 1050 V (300 V/cm). Injection: 1 s (S to SW) at 1050 V. $E(E_1) = +750$ mV; $E(E_2) = -100$ mV.

large increase in the number of interferences detected. The relative response at the two electrodes, along with migration time, can also be used to verify peak identity. This is particularly significant because this can be accomplished with a sample only a few nanoliters in volume containing attomole amounts of analyte.

Several dual-electrode detectors have been reported for CE[28−32] and have been used for the detection of a number of biologically important compounds such as catecholamines, thiols, disulfides, and phenolic acids. One of the major roadblocks to the implementation of dual-electrode detection for CE has been the difficulty in aligning two electrodes at the end of a fused-silica capillary. Ring disk microelectrodes, wires, metal tubes, and sputtered-gold electrodes have all been used as components of dual-electrode detectors for conventional CE. Dual-electrode detection for CE is much simpler to implement in the microchip format. Electrodes can simply be deposited on the glass substrate in the desired configuration. Alignment of the electrodes with the channel imprinted in the PDMS is accomplished with a simple light microscope.

The electrochemical detector described above was evaluated for dual-electrode detection. The electrode plate contained four working electrodes spaced approximately 40 μm apart (Figure 1). Dual-electrode detection was performed by applying the appropriate potentials to the first two electrodes in the microband array positioned just outside the separation channel. Figure 4 shows a detection of tyrosine, HIAA, and catechol following CE separation. All three compounds are detected at E_1, as all the compounds undergo oxidation at +750 mV. However, only catechol forms a stable oxidation product that is reduced at E_2 (−100 mV).

Collection efficiencies were determined for compounds that undergo chemically reversible redox reactions. Collection efficiency can be defined as the ratio of the cathodic and anodic currents and is dependent on many factors including the electrode surface area, the distance between the electrodes, and the position of the electrodes with respect to the separation channel.[40] The collection efficiencies for catechol were determined at several

Table 1. Collection Efficiency vs Field Strength for 200 μM Catechol Diluted in Water

field strength (V/cm)	N_e (%)[a]	RSD (n)	field strength (V/cm)	N_e (%)[a]	RSD (n)
200	28.7	0.9 (6)	350	27.0	0.4 (4)
250	27.2	0.2 (3)	400	25.9	0.6 (6)
300	26.4	0.8 (6)			

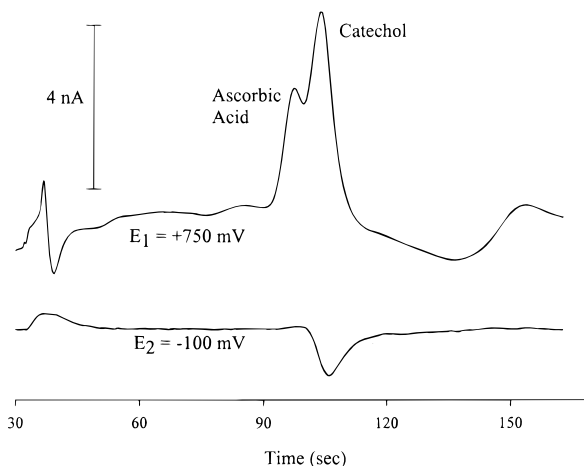[a] Effective collection efficiency. $E(E_1) = +780$ mV; $E(E_2) = -100$ mV.

Figure 5. Dual-electrode detection of an unresolved mixture of 100 μM ascorbic acid and catechol. Separation conditions: 25 mM boric acid, pH 9.2; applied potential 870 V (250 V/cm). Injection: 1 s (S to SW) at 870 V. $E(E_1) = +750$ mV; $E(E_2) = -100$ mV.

different field strengths (Table 1) and found to decrease slightly, from 28.7 to 25.9%, as the separation voltage was increased from 200 to 400 V/cm. This was expected because, at lower field strengths, mass transport across the electrodes is slower, resulting in higher collection efficiencies. These collection efficiencies are similar to those obtained for conventional CE-EC dual-electrode systems.[32]

Dual-electrode detection in the series configuration can be useful for identifying peaks of complex mixtures. Figure 5 shows the electropherograms of an unresolved mixture of ascorbic acid and catechol. Without dual-electrode detection, it is difficult to assign peak identity. Intuitively, one would expect ascorbic acid to migrate later than catechol, on the basis of its mass and charge. The electropherogram generated from E_2 reveals that catechol migrates slightly later than ascorbic acid, indicating that borate complexation is playing a role in the mobility of catechol. The borate complex of catechol is negatively charged, resulting in a slightly longer migration time than that of the native compound.[41] This assignment was confirmed by the lack of a response at E_2 following injection of an ascorbic acid standard. In this case of extremely close migration times, dual-electrode detection provides confirmatory evidence of peak identity.

CONCLUSION

The integration of electrochemical detection with PDMS-based microchip CE devices has been described. This type of device

(40) Roston, D. A.; Shoup, R. E.; Kissinger, P. T. *Anal. Chem.* **1982**, *54*, 1417A−1434A.
(41) Landers, J. P.; Oda, R. P.; Schuchard, M. D. *Anal. Chem.* **1992**, *64*, 2846−2851.

allows the rapid prototyping of CE channels in PDMS while permitting the use of standard photolithographic procedures to construct the electrode plates. The reversible bonding procedure does not damage the electrodes and also makes it possible to easily clean the PDMS layer if the channels become blocked. The use of dual-electrode detection in the series mode was shown to be useful for monitoring chemically reversible redox reactions as well as for identifying peaks of unresolved mixtures. Future work will focus on biological applications of PDMS-based microchip CE-EC devices, including the detection of Cu(II)−peptide complexes, electrochemically active derivatives of substance P, and DNA.